SEARCHING FOR MICROBES AND OTHER BIOREMEDIATION TOOLS

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Introduction

The National Passenger Railroad Corporation (Amtrak) is in the process of selecting bioremediation tools to enhance our waste water treatment systems. The primary contaminants of our waste water is diesel fuel, lube oil, and gear lubricants. The goal is to have a tool capable of being used at any Amtrak location in the United States. The bioremediation products are tested on a bench scale in Washington, D. C. and pilot tested in Los Angeles. Los Angeles is the chosen test site due to its mild climate and infrequent rain. The waste water treatment system in Los Angeles is a series of three settling ponds with a belt oil skimmer.

Experimental

The bench scale testing was performed using the following materials: a 10-gallon aquarium, a small air pump, a thermometer, 3 gallons of tap water, 20 drops of waste water from the Wilmington facility, a plastic teaspoon, a screen, a pipette, one 16 ounce glass bottle, and ½ mug of sea salt. Microbes are supplied for these experiments by various vendors.

- The procedure is to aerate the aquarium filled with the tap water for approximately 1 hour.
- 2. Add 20 drops of the waste water to the aquarium.
- Fill a 16 oz glass bottle with a waste water sample from the aquarium and place it in a cooler for analysis.
- 4. Empty the aquarium.
- 5. Repeat steps 1 and 2.
- Spray ¼ teaspoon of microbes through the screen over the waste water in the aquarium.
- 7. Conditions: Temperature 74° F and pH 9.5.

Repeat the experiment using fertilizer and a greater concentration of waste water.

- Aerate aquarium for 2 hours.
- Fill 16 oz glass bottle with 100 drops of waste water and tap water. Place in the cooler for analysis.
- 3. Place 100 drops of waste water in a plastic tubing in the aquarium.
- 4. Filtered fertilizer into the tank using a mesh screen.
- 5. Spray ¼ teaspoon of microbes through screen over oil in the tubing.
- Place a foam partition between tubing and air pump to eliminate over spill at the tubing.
- 7. Conditions: Temperature 72° F and pH 9.5.

The pilot testing was performed using the following materials: three 2lb. bags of bacteria, three 2 lb. bags of nutrients, nine 55-gallon drums of catalyst, Microbes are supplied for these experiments by various vendors. The products were introduced into three settling ponds 52 feet by 52 feet and 8 feet

deep. The three ponds were identified as S-1, S-2, and S-3. S-1 was filled to capacity, S-2 was ½ full, and S-3 was ½ full.

- 1. Add three 55- gallon drums of catalyst per pond.
- Hydrate one bag of nutrients with three gallons of water and then add one container per pond.
- Hydrate one bag of bacteria with three gallons of water and then add one container per pond.
- 4. Conditions: Temperature 67° F.

No aeration or agitation was performed. All products were poured into the waste water. The waste water settling ponds had been inactive for several months prior to the pilot test (no treatment was being performed). The pilot test was performed for a period of five months. Analysis was performed monthly for the entire five months and samples were taken from the three phases of each pond top, middle, and bottom. The samples were analyzed for total petroleum hydrocarbons diesel specific EPA Method 8015 (TPH-D), metals EPA Method 6010, total recoverable petroleum hydrocarbons EPA Method 418.1, and oil and grease EPA Method 413.1. The analysis was performed by a California State ELAP certified laboratory.

Results and Discussion

The results from the first bench test showed after 24 hours oil flakes observed and a slick surface. A large amount of debris settled to the bottom of the aquarium. After 6 days the oil layer becomes thinner but the slick surface remains. More debris settles at the bottom. After 10 days the oil layer is even thinner and more debris has settled at the bottom of the aquarium. After 6 days the oil layer becomes thinner but the slick surface remains. The settled debris at the bottom of the aquarium increases. After 10 days the oil layer is even thinner and more debris has settled at the bottom of the aquarium.

The second bench test was done introducing the fertilizer to increase microbial activity. The analytical results are shown below: Table 1 Bench Testing Results

Before	After	Analytical Method
692 mg/L	102 mg/L	8015
3.340 mg/L	1,840 mg/L	418.1

There was a color change after 24 hours from black to brown. The slick surface condition was observed again. The debris at the bottom of the aquarium was also observed again. The results listed as after above or from 48 hours of treatment with the microbes. The removal efficiency was calculated at 85% for the diesel fuel and 44% for the total petroleum hydrocarbons.

The pilot test results are very similar to what we observed in the bench test. The only real difference is that a longer period of time was allowed for the microbes to perform. See attached figures for the results for S-1, S-2, and S-3. Month 1 Observations

- S-1 appears to have a larger amount of oil rising to the surface. The oil is thicker and darker than before.
- S-2 has a huge algae bloom which covers the surface of this pond.
- S-3 has an algae bloom and the oil layer looks thinner.

Month 2 Observations

- S-1 appears to have a larger amount of oil rising to the surface. The oil is thicker and darker than before.
- S-2 still has some algae and the surface color is changing from black to brown.
- S-3 has more and surface color is changing from black to brown.

Month 3 Observations

- S-1 surface color is changing from black to brown.
- S-2 has no algae growth and the surface appears much clearer you can see to the bottom of the pond. The sludge can be clearly seen.
- S-3 has no algae growth and the surface appears much clearer you can see to the bottom of the pond. The sludge can be clearly seen.

Month 4 Observations

All ponds appear to be clear at the surface. The sludge looks darker and can be seen very clearly.

The analytical test results show a c decrease in the surface oil in all three ponds. The most oil contaminated pond S-1 showed the largest decrease in diesel and other petroleum hydrocarbons. See the attached Figures 1-6.

Conclusions

Bench testing of microbes can be correlated to pilot scale testing under industrial scale conditions. In both tests the microbes decreased the amount of surface oil in the waste water over time. The larger the amount of oil contamination the greater the activity of the microbes in degrading the oil. The efficiency of the microbes was observed in all cases to be greater than 80%. The time was controlled in these experiments and perhaps that had an impact on microbe efficiency. The additional time would probably cause this to increase.

The increased soil hydrocarbon numbers was not caused by surface oil migrating to the sludge phase. The more likely conclusion is that microbes migrated to the sludge phase after all of the surface oil was degraded. The beginning of the sludge degradation is observed with a large initial increase in petroleum concentration and then a tapering. If the pilot test had been maintained longer a decrease in the sludge petroleum concentrations should be observed. In the future, tests will be conducted with new microbes using the same methods on waste water. There will also be additional tests performed on soil and sludge under similar conditions.

The success of bioremediation tools is very important to the railroad industry. A reasonably priced treatment technology can save thousands of dollars in disposal costs. The disposal costs including treatment and transportation for fuel contaminated soil is generally between \$26.00-\$35.00 per ton. The price to treat soil using bioremediation methods is approximately \$2-\$10 per ton. Bioremediation of water has similar costs comparison that is why it is so important to have test methods that prove which products are really successful.

References

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Figure 1 Pond S1

S1-Top	S-1-Middle	S-1-Bottom
1800	23	8400
1300	11	49,000
11	9.4	42.000

Figure 2 Pond S2

S-2-Top S-2-Middle		S-2-Bottom
8.9	6	2500
5.1	181	21,000
5.1	5.1	29 000

Figure 3 Pond S3

S-3-Top S-3- Middle		S-3-Bottom
0	0	7.8
3	3	5,000
3	4	4.300

Figure 4

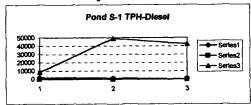


Figure 5

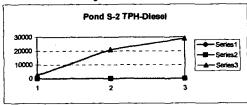


Figure 6

